AD_____

Award Number: DAMD17-99-1-9326

TITLE: 99-Technetium Sestamibi Scanning to Predict the Efficacy of Estramustine Phosphate in Overcoming Paclitaxel Resistance in Patients with Advanced Breast Cancer

PRINCIPAL INVESTIGATOR: Matthew D.Volm, M.D.

CONTRACTING ORGANIZATION: New York University School of Medicine
New York, New York 10016

REPORT DATE: September 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 01 - 31 Aug 02)

4. TITLE AND SUBTITLE
99-Technetium Sestamibi Scanning to Predict the Efficacy of Estramustine Phosphate in Overcoming Paclitaxel Resistance in Patients with Advanced Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9326

6. AUTHOR(S) :
Matthew D.Volm, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York University School of Medicine
New York, New York 10016

E-MAIL:
Matthew.volm@med.nyu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

This research investigates the ability of 99-Technetium Sestamibi (Tc-99-SM) to serve as a non-invasive means of assessing the presence of clinically relevant drug resistance in patients with advanced breast cancer. Tc-99-SM is a substrate of p-glycoprotein (P-gp), the transmembrane drug efflux transporter involved in classic multi-drug resistance (MDR). We hypothesize that that rapid clearance of Tc-99-SM correlates with the presence of functional multi-drug resistance and can be used to predict which patients will have tumors resistant to drugs that are MDR substrates. We have demonstrated marked variability in the tumor clearance of Tc-99-SM among patients. The second stage of our work is to conduct a clinical trial to determine whether changes in 99-Tc-SM clearance following the administration of an MDR inhibitor can predict effectiveness of the inhibitor in overcoming drug resistance. We have met with difficulty in obtaining an MDR inhibitor appropriate for use in the study, as recent studies have cast doubt on the ability of estramustine to reverse MDR, and biricodar, our second choice, is no longer being manufactured. Recently, however, compelling laboratory studies have shown that the agent ZD1839 (Iressa) is a highly potent inhibitor of P-gp and other drug efflux transporters likely to be significant mediators of drug resistance in breast cancer. ZD1839 is expected to be an important anti-cancer agent in the coming decade, and using it to test our hypothesis in a clinical trial will provide valuable information. We have therefore rewritten the clinical protocol to reflect the use of ZD1839 as the MDR reversing agent and have received approval from our IRB for this study and are awaiting approval from the FDA and the DOD's Human Subjects Research Review Board.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
breast cancer	6
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

The purpose of this research is to investigate the ability of 99-Technetium Sestamibi (Tc-99-SM) to serve as a non-invasive means of assessing the presence of clinically relevant drug resistance in patients with advanced breast cancer. Tc-99-SM is a substrate of the p-glycoprotein, the transmembrane drug efflux transporter involved in classic multi-drug resistance (MDR). We are testing the hypothesis is that rapid clearance of Tc-99-SM correlates with the presence of functional MDR and can be used to predict which patients will have tumors resistant to chemotherapy drugs that are MDR substrates. More importantly, we are investigating whether changes in the tumor clearance of 99-Tc-SM observed before and after the administration of an MDR inhibitor, can predict whether the inhibitor can overcome clinical drug resistance in an individual patient.

Body

Task 1: *Complete a clinical trial of estramustine/paclitaxel in patients with advanced cancer of the breast refractory to paclitaxel, Months 1-30:*

- *Finalize clinical protocol. Obtain Institutional Review Board approval*
- *Recruit patients from the clinics of Bellevue and Tisch Hospital who have advanced breast cancer and are candidates for treatment with paclitaxel. Initiate treatment with paclitaxel.*
- *At the time each enrolled patient demonstrates resistance to paclitaxel, begin estramustine/paclitaxel. Patients may demonstrate primary resistance to paclitaxel (no response to an adequate trial of paclitaxel) or secondary resistance (failure following an initial response to paclitaxel).*

We have encountered unexpected difficulties performing Task 1. During the process of finalizing the research protocol, new information about the interaction between the estramustine and p-glycoprotein became available. Specifically, a study of the pharmacokinetics of paclitaxel given concurrently with estramustine indicates that estramustine does not inhibit p-glycoprotein or otherwise affect drug efflux from tumor cells (1). While this clinical finding is at odds with prior laboratory studies indicating an inhibitory effect of estramustine on drug efflux (2,3), it strongly casts doubt on the ability of estramustine to serve as a clinical inhibitor of MDR. We therefore investigated the use of other agents that are more likely to successfully inhibit drug efflux and decided to replace estramustine with the biricodar dictrate (VX-710, Incel™) as the MDR inhibitor for purposes of this study. The protocol was approved by the Institutional Review Board at New York University and the Surgeon General's Human Research Review Board. Unfortunately, as final preparations were being made to enroll patients on the protocol, Vertex pharmaceuticals ceased manufacturing biricodar, and we were unable to obtain a supply of drug to go forward with the study.

We have investigated other MDR inhibitors that might be used to investigate the utility of Tc-99-SM scanning as a mean of predicting clinical benefit from an MDR inhibitor in taxane-resistant breast cancer. Recent laboratory studies indicate that an important new agent, ZD1839 (Iressa) has a profound inhibitory effect on P-glycoprotein (classic MDR) and breast cancer related protein (BCRP), a drug efflux protein that may be particularly important in the development of drug resistance (personal communication, Dr. Peter Houghton). Studies in animal tumors have demonstrated that that ZD1839 is very effective at synergistic the activity of a variety of

chemotherapy drugs, including paclitaxel. Interestingly, in these experiments the enhanced anti-tumor activity achieved by adding Iressa to chemotherapy did not depend on the tumor's level of EGFR expression (4), suggesting that mechanisms other than EGFR inhibition, such MDR reversal, may be playing an important role. ZD1839 is expected to be an important anti-cancer agent in the coming decade, and using it to test our hypothesis in a clinical trial will provide valuable information. We have rewritten the clinical protocol to reflect the use of ZD1839 as the MDR reversing agent in the study, and the study has been approved by the NYU IRB. Extensive revisions to the protocol and consent form were requested by both the DOD and Astra-Zeneca, the manufacturer of ZD1839; these revisions have been incorporated into the protocol and consent form. The protocol is awaiting final approval from the FDA and the DOD's Human Subject Research Review Board. We have requested a "no cost" extension from the DOD in order to complete this project using ZD1839 as the MDR inhibitor.

Task 2: *Concurrently with Task 1, complete an imaging study evaluating serial Tc-99-SM scanning to assess the presence of functional drug efflux at three critical time points in the treatment of patients during the clinical trial described in Task 1, Months 1- 30:*

- *Baseline Tc-99-SM scans will be performed before the administration of therapy with paclitaxel.*
- *At the time each patient exhibits resistance to paclitaxel, before the administration of estramustine, a second Tc-99-SM scan will be obtained.*
- *Following the administration of the first 3-day treatment with estramustine, a third Tc-99-SM scan will be obtained.*

An imaging study with Tc-99-SM scanning has been approved by the Institutional Review Board at New York University and by the Surgeon General's Human Research Review Board. Under this study, we have performed Tc-99-SM scanning in 3 patients with advanced breast cancer. We have carefully analyzed the Tc-99-SM clearance data, and have found significant variability in the rate of clearance of Tc-99-SM from the patients' tumors. We believe that this represents varying degrees of expression of relevant drug efflux proteins (p-gp and/or MRP) in these patients. Because of the problems with obtaining an MDR inhibitor, the next steps in our project are to determine whether ZD1839 , administered in the clinical trial described in Task 1, can significantly increase tumor retention of Tc-99-SM, and whether the change in retention is reflected clinically as reversal of drug resistance to paclitaxel.

Task 3: *Data analysis and report of conclusions Months 31-36:*

- *Evaluate correlations between tc-99-SM clearance, response to paclitaxel, and the efficacy of estramustine in overcoming paclitaxel resistance.*
- *A report of the conclusions and an initial manuscript will be prepared.*

Because of the need to rewrite the protocol to reflect a new MDR inhibitor, there is not yet data to complete Task 3.

Key Research Accomplishments

We have performed preliminary studies of Tc-99-SM scanning in patients with advanced breast cancer and found variability in the clearance of Tc-99-SM suggesting that altered drug efflux may be a significant mechanism of drug resistance in some patients.

Reportable Outcomes

There are not yet reportable outcomes from this work.

Conclusions

At present, our conclusions are limited. Consistent with our hypothesis that the rate of Tc-99-SM clearance reflects the expression of drug efflux proteins, we have observed significant intrapatient variation in the studies of tumor clearance of Tc-99-SM. We have met with difficulty in obtaining an MDR inhibitor appropriate for use in the study, as recent studies have cast doubt on the ability of estramustine to reverse MDR, and biricodar, our second choice, is no longer being manufactured. Recently, however, compelling laboratory studies have shown that the agent ZD1839 (Iressa) is a highly potent inhibitor of P-gp and other drug efflux transporters likely to be significant mediators of drug resistance in breast cancer. ZD1839 is expected to be an important anti-cancer agent in the coming decade, and using it to test our hypothesis in a clinical trial will provide extremely valuable information. We have therefore rewritten the clinical protocol to reflect the use of ZD1839 as the MDR reversing agent in the study. The study has been approved by our IRB, and is awaiting final approval by the Surgeon General's Human Subject Research Review Board. We have requested a "no cost" extension from the DOD in order to allow us to complete project.

References

1. Garcia A, Keren-Rosenberg S, Parimoo D, Muggia F. Phase I and pharmacologic study of estramustine phosphate and short infusions of paclitaxel in women with solid tumors. J Clinical Oncology 1998; 16:2959-2963.
2. Speicher L, Barone L, Chapman A, et al. P-glycoprotein binding and modulation of the multidrug-resistant phenotype by estramustine. Journal of the National Cancer Institute 1994; 86:688-94.
3. Yang C, Shen H, Horwitz S. Modulation of the function of P-glycoprotein by estramustine. Journal of the National Cancer Institute 1994; 86:723-5.
4. Sirotnak FM. Zakowski MF. Miller VA. Scher HI. Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clinical Cancer Research.* 2000;6(12):4885-92.